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Organic *N*–Chloramines: Chemistry and Toxicology

by Frank E. Scully, Jr.* and Maxwell A. Bempong†

The stability of aqueous solutions of organic *N*-chloramines, suspected of contaminating chlorinated water, has been studied. Two factors influence the decomposition of solutions of *N*-chloropiperidine and *N*-chlorodiethylamine: a spontaneous decomposition and photodecomposition. Since solutions of these compounds are relatively long-lived, a need for an analytical method for their identification is discussed. A new method is described which involves reaction of organic *N*-chloramines with arenesulfinic acid salts. The method gives high yields of stable arenesulfonamides. Several toxicological studies of *N*-chloropiperidine are described. The compound is mutagenic by Ames assay in *Salmonella typhimurium* strain TA 100 and does not require metabolic activation as indicated in a total body fluids analysis using C57BL/J6 mice. *N*-Chloropiperidine was subjected to a modified *in vitro* cell transformation assay using diploid fibroblast cells from Syrian hamster fetuses. A maximum number of foci of 4 per dish was observed at a seeding of 5×10^3 cells/60 mm dish. Under similar conditions, MNNG-induced foci ranged from 4 to 7 per dish.

Introduction

For almost seventy years chlorination of water has been viewed as a public health breakthrough. Recently, however, the observation that some organic by-products of water chlorination produce tumors in laboratory animals (*1,2*) has led to concern about the health effects of water chlorination and more extensive investigation of alternate methods of disinfection.

Many hundreds of organic compounds have been identified in drinking water. However, these represent only a small fraction of the total organic material present.

Routine analysis of residual chlorine in water and wastewater includes an analysis of chloramines, yet no distinction is made between organic and inorganic *N*-chloramines because of the lack of suitable analytical method. Organic *N*-chloramines, as a class, are unusually thermally labile. While some chloramines are distillable, most undergo rapid exothermic decomposition on all attempts at isolation even at room temperature. Some decompose explosively. Therefore, gas chromatographic separation or gas chromatographic/mass spectrometric identification procedures are precluded.

However, organic nitrogen compounds are abundant and essential components of nature. They occur as proteins, amino acids, nucleic acids, and biogenic amines. In addition, free aliphatic amines have been identified in foods including cheese (*3*), fish (*4,5*), beer (*6*), tea (*7*) and milk (*8*).

Among the amines most commonly identified are methylamine, dimethylamine, and ethylamine, as well as three, four, and five carbon aliphatic amines including the heterocyclic amines, piperidine and pyrrolidine. Piperidine and propylamine have been found in drinking water (*9*). Piperidine, pyrrolidine and dimethylamine have been identified in human blood and urine (*10,11*). In fact, Blau has reported that the mean basal excretion in the urine of normal human males over 24 hr is 17 mg of dimethylamine, 15 mg of pyrrolidine, and 5 mg of piperidine.

On chlorination in water, these amino compounds are converted rapidly (*12*) and essentially quantitatively (*13*) to their *N*-chloro derivatives. Therefore a discussion of nitrogenous organic compounds in chlorinated water must be directed toward organic *N*-chloramines and *N*-chloroamino acids rather than

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free amino compounds. The fact that organic *N*-chloramines have not been identified in chlorinated water raises two questions: are the organic *N*-chloramines which are formed short-lived and, if they are long-lived, how can they be identified? This paper will address these issues.

In addition we have begun to examine the toxicology of organic *N*-chloramines. The fact that many *N*-chlorodialkylamines are readily synthesized and can be isolated pure makes them attractive candidates for these studies. *N*-Chloropiperidine, the *N*-chloro derivative of an amine that has been found in drinking water, has been the subject of much of our recent work.

Experimental

Stability of Organic *N*-Chloramines

Details of the experiments are described elsewhere (*14*). The aqueous decomposition of *N*-chloropiperidine at pH 7.0 has been repeated at $10^{-3}M$ and $10^{-4}M$ concentrations using a Cary 219 UV/VIS spectrophotometer. The sample chamber was thermostated to 25.0 ± 0.2°C. The instrument was programmed to take an absorbance measurement every 90 min and in the interval remove the sample from the light path. Readings were taken over 60 hr. A plot of ln absorbance versus time gave a straight line of slope 0.0087 ± 0.0012 sec^{-1}. From this the half-life was calculated.

Derivatization of Organic *N*-Chloramines

N-chloropiperidine was synthesized by the method previously described (*15*). *N*-Chlorodimethylamine and *N*-chlorodiethylamine were synthesized by adding below 5°C the appropriate amine to a slight excess of a standardized commercial solution of sodium hypochlorite (Chlorox) that had been saturated with salt. After stirring for 10 min, the chloramine was separated from the water in a separatory funnel. *N*-Chlorodimethylamine was purified by drying over freshly activated molecular sieves and distilling on a high vacuum line. *N*-Chlorodiethylamine was dried over anhydrous calcium chloride and distilled at atmospheric pressure. The chloramines were either used immediately or stored in the dark in a freezer.

A solution of 40 mmole of either benzenesulfinic acid sodium salt (Aldrich) or *p*-toluenesulfinic acid sodium salt (Aldrich) in deionized water (100 ml) was stirred in an ice bath. The pure chloramine (20 mmole) was added dropwise over 15 min. Although the sulfonamide formed immediately the product mixtures were routinely stirred overnight to allow all of the sulfonamide to crystallize. The sulfonamides were filtered and dried under vacuum. All three sulfonamides were obtained as white solids requiring no further purification. They each had infrared and NMR spectra, melting points and mixed melting points identical with the same sulfonamides prepared by reaction of the amine with the appropriate arenesulfonyl chloride in basic solution. The yields are found in Table 1.

Table 1. Derivatization of organic *N*-chloramines.

Chloramine	Derivative	Yield, %
N-Chlorodimethylamine	*p*-Toluenesulfonamide	100
N-Chlorodiethylamine	*p*-Toluenesulfonamide	63
N-Chloropiperidine	Benzenesulfonamide	78

Alternatively, amine (20 mmole) and sodium arenesulfinate (40 mmole) were dissolved in deionized water (100 ml). Glacial acetic acid (20 mmole) was added as a buffer and to help dissolve less water soluble amines. The solution was chilled in an ice bath below 10°C and stirred as sodium hypochlorite (30 mmole) was added dropwise over a period of 1 hr. The resulting mixture was stirred overnight at room temperature, filtered, and the sulfonamide dried in a vacuum desiccator. In this manner the benzenesulfonamide derivative of *N*-chloro-*sec*-butylamine was obtained pure in a 95% yield. Similarly, the benzenesulfonamide derivatives of the following *N*-chloramines were obtained pure after recrystallization (pure yield, recrystallization solvent): *N*-chloroaniline (85%, ethanol/water), *N*-chloro-L-leucine (78%, ethanol/water), and *N*-chloroglycine (70%, water). Inorganic chloramine gave an 89% yield of crude benzenesulfonamide which was purified by recrystallization from ethanol/water. *N*-Chloropyrrolidine gave a 100% yield of its *p*-toluenesulfonamide derivative. The *N*,*N*′-bisbenzenesulfonamide derivative of *N*,*N*′-dichloroethylenediamine was prepared in 81% pure yield (recrystallization from ethanol) by the procedure above and the following molar ratios: ethylenediamine (25 mmole), sodium benzenesulfinate (100 mmole), and sodium hypochlorite (100 mmole).

The infrared and NMR spectra, as well as melting points and mixed melting points, were identical in every case to the authentic compound prepared by the reaction of the amine with the appropriate arenesulfonyl chloride in basic solution.

Mutagenicity Studies

Details of the methods used in the toxicity and mutagenicity studies of *N*-chloropiperidine have been described in detail elsewhere (*16*).

Cytogenicity Analysis

Details of the methods used in the cytogenicity studies have been described in detail elsewhere (*16*).

In Vitro Cell Transformation Assay.

The technique of DiPaulo et al. (*17*) was used with primary fibroblast cells from Syrian hamster embryos with the modification described in the text. Details have been described elsewhere by us (*18*).

Results and Discussion

Stability of Organic *N*-Chloramines

Recently we reported a study of the aqueous decomposition of *N*-chloropiperidine (NCP) and *N*-chlorodiethylamine (NCDEA) (*14*). The following main points emerged. NCP and NCDEA are considerably longer lived compounds (3.3 days and 2.2 days, respectively, at 25°C at pH 7.0) than the *N*-chloroamino acid, *N*-chloroalanine (55 min), calculated from the data of Stanbro and Smith (*19*). The half-lives of all three compounds do not change from pH 3.5 to pH 9, but increase dramatically below pH 3.0.

Early in our study we found when we followed the NCP concentration by continuously monitoring its absorbance maximum at 262 nm that we photodecomposed it in the sample chamber of the spectrophotometer. Subsequently, special precautions were taken to ensure that photodegradation did not take place in the sample chamber of the UV/VIS spectrometer. To overcome this problem measurements were taken intermittently and the solution removed from the sample beam between measurements. Such a precaution is suggested for all spectrophotometric measurements on chloramines.

The photodecompositions of NCP and NCDEA follow a much different pH profile than their aqueous decomposition. They are most stable at high pH's and photodecomposition rates increase dramatically going from pH 9 to 7 to 5 and lower (Fig. 1). The products of the reaction are imines. NCDEA for instance gave ethylidene ethylamine and acetaldehyde as main products of the photoreaction at pH 7.0 [see Eq. (1)].

(1)

Extrapolation of our results to real environmental situations is difficult without more knowledge of the concentrations of organic *N*-chloramines and *N*-chloroamino acids in chlorinated water. We may conclude that organic *N*-chloramines do undergo a natural first-order decomposition (probably to imines) in water and that their decomposition in the environment is enhanced by ultraviolet light. Finally, under similar conditions of concentration, temperature, and light, *N*-chloramines are much longer lived than *N*-chloroamino acids.

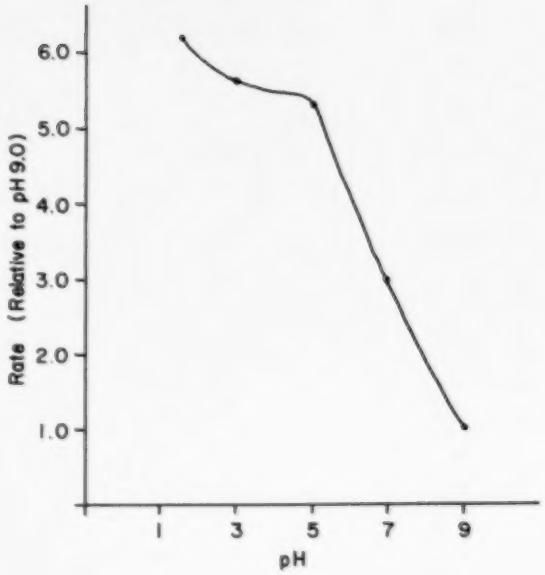

FIGURE 1. Relative rates of photodecomposition of *N*-chlorodiethylamine at varying pH.

Derivatization of Organic *N*-Chloramines

Because of their thermal lability, organic *N*-chloramines are not likely to withstand the harsh conditions of concentration, gas chromatographic separation and mass spectrometric identification. Therefore, if direct isolation is precluded, derivatization is required.

Recently we reported a reaction of organic *N*-chloramines with sodium salts of arenesulfinic acids [Eq. (2)] (*20,21*). The reaction gives good to high yields of stable arenesulfonamides at or below room temperature as shown in Table 1.

(2)

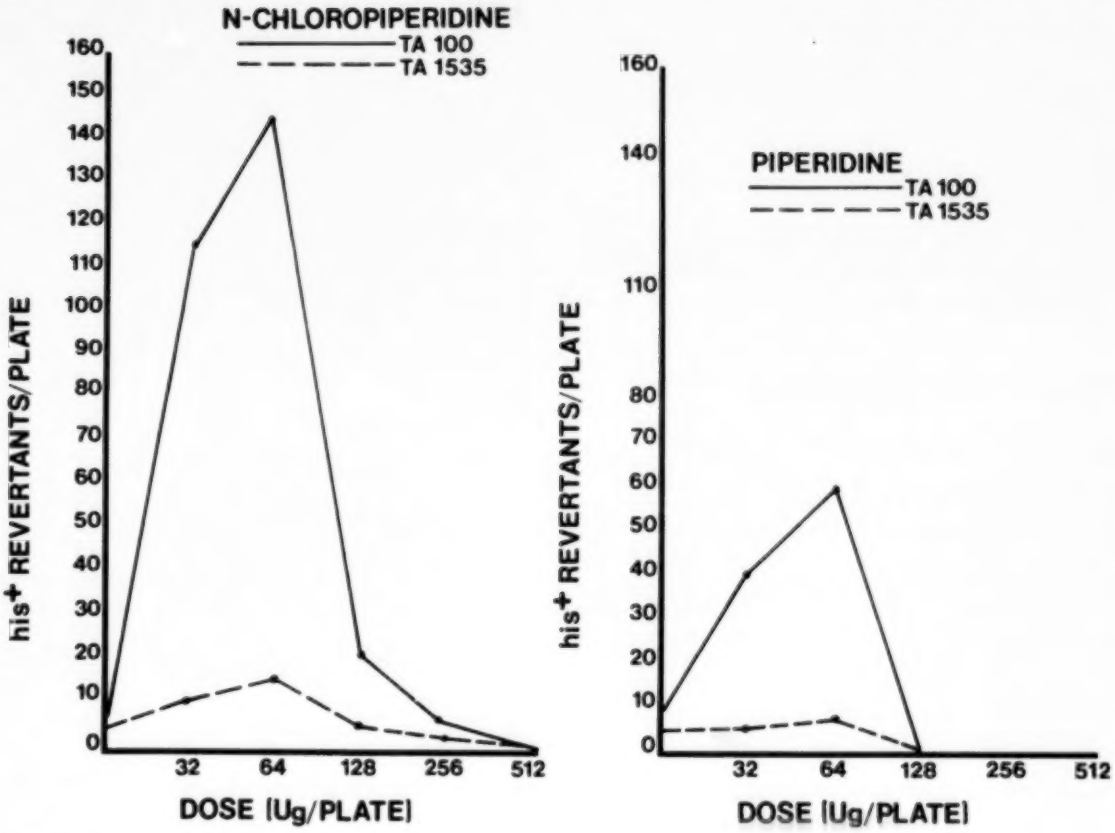

FIGURE 2. Dose-effect relationships for *N*-chloropiperidene and piperidine induction to histidine revertants in TA 100 and TA 1535 Salmonella tester strains.

Many chloramines cannot be distilled and isolated pure the way the three listed in Table 1 can. However, they can be generated in aqueous solution by reaction of the amine with sodium hypochlorite. This can be done either in the presence of the sodium arenesulfinate salt or the chloramine can be generated first and the sulfinate salt added subsequently. In this way, inorganic chloramine (89%) and *N*-chloro derivatives of pyrrolidine (100%), *sec*-butylamine (95%), aniline (85%), and ethylenediamine (81%), as well as others, could be derivatized in good yield. The method is also applicable to the *N*-chloro derivatives of the amino acids L-leucine (78%) and glycine (70%). The examples illustrate the broad scope of the reaction.

Although the derivatizations described here were performed for ease of isolation and product identification on amine or chloramine solutions of approximately 0.2*M*, we are currently extending these studies to dilute solutions.

Toxicological Implications of Chloramine Chemistry

Organic *N*-chloramines have a labile electrophilic nitrogen which can undergo nucleophilic substitution as illustrated, for example, in the derivatization reaction just discussed. In the classical work of Miller and Miller, an electrophilic nitrogen has been invoked as the "ultimate carcinogen" responsible for alkylamination of DNA in the mechanism of the carcinogenic action of aromatic amines (*22*). It has become clear in recent years that a common chemical characteristic required for the covalent bonding of many "ultimate" chemical carcinogens to DNA is their electrophilic nature. Therefore the chemical potential of chloramines suggests the need for more extensive toxicological information on them.

To date toxicological studies of organic chloramines have been scanty. The comparative lack of bactericidal properties of organic chloramines formed

on water disinfection are probably responsible for this, but because of the ubiquity of organic nitrogen compounds in natural water and wastewater as well as in food processing much more is needed.

Mutagenicity Studies

We have reported a study of the toxicity and mutagenicity of *N*-chloropiperidine (NCP) (*16*). NCP is toxic to C57BL/J6 mice with an LD_{50} of approximately 350 mg/kg. In addition, it gave a positive mutagenic response in Ames' *Salmonella typhimurium* strain TA 100 (see Fig. 2). When mice were given a single IP injection of NCP and a total body fluid analysis performed using TA 100 tester strain (see Fig. 2), no metabolic activation of the NCP was detected.

Cytogenetic Analysis

NCP is a strong cytostatic and cytotoxic compound on exposure to Chinese hamster ovary (CHO) cells. It interferes with normal chemical cytogenetic programming of CHO cells resulting in anomalous chromosome separation and nuclear distribution. Macro- and micronucleation were observed. Most importantly, NCP induces structure aberrations of the chromosomes (breaks, exchanges, fragments, ring configurations and centromeric errors), the frequency of which is proportional to the NCP concentration used (*18*).

In Vitro Cell Transformation Assay

Freshly isolated diploid fibroblast cells from Syrian hamster fetuses were used to study *in vitro* carcinogenicity of *N*-chloropiperidine (NCP). Our studies have shown that exposure of early passage cells to NCP (5 – 20 μg/ml) resulted in low cloning efficiency, when the cells were plated at 10^3/60 mm dish. The maximum number of foci at this cell density did not exceed 2. We have observed that by using higher concentrations of NCP (1-4 mg/ml) and a higher cell density (5×10^3 to 5×10^4/60 mm dishes) more transformed foci were observed; however, the number of colonies observed in NCP-treated cells was significantly below that observed when *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)-exposed cells were analyzed.

The observation that early passage hamster embryo cells do not respond very well to NCP in a transformation assay led us to develop a protocol which seems to increase the number of NCP-induced foci. We have observed that if 12- to 14-week-old hamsters (both male and female) are treated with chronic low doses of NCP *ad libitum*, and if the fetuses of the treated parents are cultured, the number of foci is increased following exposure of the cultured cells to NCP. The maximum number foci per dish originally seeded at 5×10^3/60 mm dish was 4.

The foci were characterized by multilayered, irregularly oriented cells. In the positive control assay, MNNG-induced foci ranged from 4 to 7 per dish.

Conclusion

The fact that *N*-chloropiperidine is mutagenic and transforms mammalian cells *in vitro* is hardly a basis on which to make a health assessment on compounds that have not yet been specifically identified. However, these are the first studies on any members of this class of compounds, which theory suggests contaminate chlorinated water and which should be formed *in situ* on ingestion of chlorinated water. Clearly, more toxicological information is needed on this compound and other members of its class.

As contaminants of chlorinated water, the organic chloramines are obviously considerably shorter lived than, for instance, the trihalomethanes. However, their stability varies with structure and, until actual chloramines formed in water have been identified, a true assessment of the health implications of their presence will be simply speculative.

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